ROLE OF INTERLEUKIN-8 IN THE ASSESSMENT OF INNATE IMMUNE RESPONSE IN RESPIRATORY ACUTE INFECTIONS IN BREASTFED INFANTS

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Abstract: *Acute respiratory infections are the most common pathology in infants and small children. Regarding the diagnosis set up in infectious diseases, modern medicine has been recording a growing trend, of guidance for the assessment of immune response. Immunoassay diagnostic approach is supported by recent concerns of researchers who are working hard to find and validate new biomarkers that reflect the human body's immune response to the contact with microbial agents. Also, immunological actions of breastfeeding and the importance of breastfeeding for the development of a strong immune system of the infant are illustrated by more and more recent studies and publications. In breastfed infants, who are diagnosed with acute respiratory infections, the evaluation of cytokine production is important to highlight their involvement in the imunopatogenetic mechanisms of this disease, but also to assess the infant immune response to respiratory infections. Objectives: Starting from these premises, I dosed serum levels of interleukin-8 in breastfed infants, diagnosed with acute respiratory infections with the aim to find some correlations between serum levels of interleukin-8 and demographics, type of respiratory infection, presence of personal history of respiratory infections, parental smoking. Material and Methods: The study included a total of 44 subjects who were fed with breast milk. They were divided into the study group made up of 28 infants with acute respiratory infections and the control group consisting of 16 infants without signs of acute respiratory infections, or other types of infections. Results: Serum levels of interleukin-8 were significantly higher in subjects with acute respiratory infections, compared to subjects in the control group. Conclusions: Interleukin-8 could be an immunological marker of acute respiratory infections in infants. There is a need for further research to determine if the increase in the serum level of interleukin-8 in infants with acute respiratory tract infections is the result of the immune mechanisms that occur as a body's response to the contact with infectious agents, and if breastfeeding is one of positive factors influencing this process.*

INTRODUCTION

According to the World Health Organization (WHO), respiratory tract acute infections rank first among the causes of morbidity and mortality in infants and small children, both worldwide and in our country. The classical understanding of the pathogenesis of infections supposed that disease manifestations are a direct result of microbial replication and their cytotoxicity. Although these mechanisms may be important in understanding the processes that underlie the onset and subsequent evolution of the acute infections of the respiratory tract, most research has focused on the immunological and non-immunological responses to infections, and their role in disease pathogenesis. Several scientific observations suggest that the immunological mechanisms could be the key to explain the increase of severity of viral respiratory infections in childhood, whether they are complicated with bacterial superinfection, or that evolves with the emergence of other complications.(1,2,3)

Premise: The research conducted in support of natural food and the extended period of breastfeeding of infants have created a sound scientific basis to support the "high specific protection transfer" from the mother to the breastfed infant. Also, there have been found some correlations between "the immune system in breast milk" and breastfed infant's immune status, but also with the subsequent immunological development.

Among the factors present in breast milk, with immunological, hormonal, enzymatic and trophic activities, it seems that cytokines play a significant part in immunomodulation and immune protection. Cytokines in breast milk remain as undamaged, they are immunologically active and can exercise their biological activities after arriving in the baby's gut, on the one hand due to the protection towards the digestive enzymes offered by protease inhibitors, particularly α-1 antitrypsin and α-1 antichemotripsina, which are also present in breast milk, and on the other hand, the gastric digestion of proteins is reduced in the first 3 months of life due to gastric immaturity at this age.(4,5,6)

A number of recent studies measuring the dosages of interleukin-8 (IL-8), either by ELISA technique and by variants thereof, or by various techniques of molecular biology, have recorded changes in the serum levels for these interleukins, in the acute infections of respiratory tract in infants, especially in bronchiolitis and pneumonia caused by infection with respiratory syncytial virus (RSV). Other studies have made measurements of interleukin-8 (IL-8) from the nasopharyngeal secretions or tracheobronchial aspirates harvested from infants infected with respiratory syncytial virus (RSV), bringing new evidence of the active involvement of this chemokine in the immune response in acute respiratory infections.(6,7,8,9,10)

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It is well known the active participation of interleukin 8 (IL-8) as an agent with an important for chemotactic function for neutrophils (PMN), in respiratory infections, especially in bronchiolitis given by respiratory syncytial virus (RSV). If we add to these records the evidence demonstrating the presence of interleukin 8 (IL-8) in the breast milk, which is a bioactive immunomodulator factor, as well as its transfer to the infant through breastfeeding, we could promote the IL-8 as a serological marker of the innate or non-specific immune response in respiratory infections.

Interleukin 8 (IL-8) is a protein known as "neutrophil chemotactic factor" due to its main role of neutrophil chemotaxis inducer factor. Interleukin 8 (IL-8) is the representative of chemokines, a large family of cytokines that includes proteins with relatively small molecular weight (MW), reason for which it has been also renamed CXCL8 by the "Chemokine Nomenclature Subcommittee".

From a functional perspective, interleukin 8 (IL-8) is a chemokine with mediator role of innate or non-specific immunity, and a pro-inflammatory factor. Interleukin-8 (IL-8) is secreted primarily by macrophages (MF), and other types of cells: lymphocytes, platelets, fibroblasts, endothelial cells, epithelial cells, smooth muscle cells of the respiratory tract.

Lately, a number of studies have shown the possibility of using serum interleukin 8 (IL-8) concentration as a diagnostic marker for bacterial infections. Interleukin-8 (IL-8) was found to be a quite easily accessible parameter and a prediction factor especially important in the diagnosis of early onset neonatal infections.

Also, in the innate immune response in bronchiolitis by respiratory syncytial virus (RSV), the importance of interleukin 8 (IL-8) is also underlined by genetic studies that demonstrate the intervention as a determining factor of the severity of the disease, of the genetic polymorphism linked to gene IL-8. Thus, as a result of genetic studies, the following have been shown: a) allele of IL-8 - 251A, which reflects the association between the increased expression of IL-8, with an increased risk for bronchiolitis with respiratory syncytial virus (RSV) and the risk to develop wheezing; b) allele of IL-8 - 781C/T, which is only associated with asthma, but not with RSV bronchiolitis.(11,12,13)

The harmful habits of parents about hygiene, nutrition, smoking in the home, especially maternal smoking during pregnancy contributes to the spread of severe forms of acute infections of the respiratory tract in infants and small children, but also to the emergence of secondary infections. Several studies on exogenous risk factors to the respiratory tract have showed decreased broncho-pulmonary ciliary activity in the infants exposed to cigarette smoke. But, as demonstrated by recent research, tobacco leads to inflammatory serological markers through an increase in the expression of mediators' inflammation, and acceleration of inflammatory processes.(14,15,16)

OBJECTIVES

Based on the assumptions outlined above, I have dosed serum interleukin-8 in breastfeed infants, and diagnosed with acute respiratory infections, aiming at: immunological evaluation of babies breastfed in acute respiratory infections; obtaining arguments in favour of integrating serum dosages of interleukin 8 (IL-8) in the immunological markers package of laboratory diagnosis of acute respiratory tract infections; finding correlations between serum levels of interleukin-8 (IL-8) and demographics, type of respiratory infection, the presence of respiratory medical history, parental smoking.

MATERIALS AND METHODS

To achieve the objectives, I studied a total of 44 subjects who were recruited from the patients admitted to the Pediatric Hospital of Sibiu, during a period of one year: January 2014 - January 2015. The study group consisted of infants under 2 years of age, fed with breast milk, and was divided into study group (SG) and control group (CG).

The study group (SG) has been made up of 28 infants, representing 63.63% of the total subjects in the study. They were hospitalized for various types of acute respiratory infections, and laboratory parameters were within a positive inflammatory profile.

The control group (CG) comprises a total of 16 infants, representing a percentage of 36.36% of the subjects under study. At the moment of harvesting samples, they did not show any clinical signs and symptoms of respiratory infections or other acute infections and the diagnostic markers for infection and inflammation have been normal for the age group selected.

Exclusion criteria were: a) treatment with corticosteroids or immunosuppressive in the last month, both the for the mother and the child; b) chronic diseases of mother and baby; c) malformations of respiratory and cardio-vascular systems in the infant; d) improper serum samples in terms of indications of the dosage kit, i.e., hemolyzed or lipemic specimens.

Ethical issues: to achieve this research, there were observed the ethical aspects of the Declaration of Helsinki. Thus, for the patients in the study, parental consent was obtained, and the consent was documented by signing the acceptance form, form which was approved by the Ethics Committee of the institution.

Determination of interleukin-8 (IL-8): blood sampling was done in the collecting tubes without anticoagulant, which was left at room temperature for 30 minutes for sedimentation. Then, the samples were centrifuged for 10 minutes at 1000 x g to obtain the serum, which was transferred in special tubes for refrigeration and stored at - 70^0 C. In order to determine the serum level of interleukin-8 (IL-8), I used the KRISHGEN Human IL-8 ELISA BioSystems (Cat.No: KB1070) research kit. Being a kit dedicated only for dosages of cytokine, it has not been provided with recommendations regarding the reference ranges. Therefore, after analyzing the results of studies that have been determined cytokines in healthy subjects, but which mostly were performed on adult subjects, I considered elevated values, all values > 35 pg/ml.(11,12,17) Dosages of Interleukin 8 (IL-8) have been performed on the automatic analyzer Dynex DS2, whose principle of analysis is the automated ELISA method.

Statistical analysis: for statistical processing of data, there has been used the Excel 2010 database, which allowed the application of statistical program SPSS version 21. There were applied methods of inferential statistics, both parametric tests that give valid results and were based on the mean of the variable as well as non-parametric tests, based on the median. I used the following tests: Kolmogorov-Smirnov, Mann-Whitney test, Kruskal-Wallis ANOVA, Student, Chi square test. A statistically significant difference was considered for a p-value <0.05.

RESULTS

As I also presented in the description of the work material, distribution of subjects within groups was as follows: 28 infants, i.e. a percentage of 63.63% in the study group (SG), and 16 infants, i.e. a percentage of 36.36% in the control group (CG). When comparing the study group (SG) to the control group (CG) regarding the average age of the infants (test ANOVA), there were significant differences: $p = 0.005$.

Thus, the average age for the study group was: 11.53 ± 5.6 months and for the control group of 6.5 ± 5.12 months with a range between 1 and 22 months. Regarding the gender distribution within the groups, one can say that female gender was representative of the study group, i.e. 68% for girls and 32% boys, while in the control group, the percentages were equal (50%). In the study group, distribution of the membership of a particular diagnosis group of respiratory diseases has been prevalent for the diagnosis of pneumonia. Specifically, of the 28 subjects of the study group, 17 were diagnosed with pneumonia (60.72%), 8 patients (28.57%) with acute upper respiratory tract infection and 3 patients (10.71%) with bronchiolitis.

The presence of respiratory infectious diseases in previous medical history may influence the immune status of the infant, so one of the aimed objectives was to see whether these issues can be correlated with serum levels of interleukin 8 (IL-8). Within the groups, there were not significant differences in examining the distribution according to the presence/absence of respiratory infections history. In both groups, the proportion of subjects who had no previous history of respiratory infections was greater, i.e. 60.71% in the study group and 68.75% in the control group.

Another aspect aimed at in this study, which may influence the immune status of the infant, and therefore that could be correlated with cytokine production in the respiratory infection is the exposure to smoking. Starting from these premises, I studied firstly the distribution within groups depending on infants' exposure to tobacco as a result of one parent or both smoking. According to the exposure source, I have formed four subgroups: mother (M), father (F), Mother and Father (FT), without exposure (NO).

Table no. 1. The distribution by gender, average age, diagnosis of respiratory infection, presence/absence of respiratory infections history, presence/absence of exposure to smoking

Demographic and clinical criteria		Study group	Control group
Distribution of subjects in the two groups (No./%)		28 infants (63.63%)	16 infants (36.36%)
Average age of subjects (months)		11.53±5.6 months	6.5±5.12 months
Gender distribution: M (No. of boys/%): F (No. of girls/%)		(9 / 32%): (19 / 68%)	(8 / 50%): (8 / 50%)
Diagnosis of respiratory infection: No./%	Pneumonia	17 infants (60.72%)	
	Acute upper respiratory infections	8 infants (28.57%)	
	Bronchiolitis	3 infants (10.71%)	
Presence of respiratory infections history: No./%		11 (39.29%)	5 (31.25%)
Exposure to smoking: No./%	Mother (M)	4 (14.29%)	3 (18.75%)
	Father (F)	8 (28.57%)	2 (12.50%)
	Mother, Father (MF)	5 (17.86%)	0 (0.00)
	No exposure (NO)	11 (39.29%)	11 (68.75%)

In table no. 1, there are presented for the study group and control group: distribution of subjects within groups, distribution by gender, average age, the diagnosis of respiratory infection, presence/absence of respiratory infections history and the distribution depending on the presence / absence of exposure to smoking.

Following the measurement of serum interleukin 8 (IL-8), there have been obtained much elevated values for infants in the study group, compared to values obtained for infants in the control group. For the average value of interleukin 8 (IL-8) and standard deviation (SD), statistical processing of the data revealed statistically significant differences only between the two groups, and no statistical significance between genders. All these results are suggestively shown in table no. 2.

Table no. 2. The average value of interleukin 8 (IL-8) and standard deviation (SD) in the study and control groups and in both genders

Group	Study group	Control group	P**
Interleukin 8 (IL-8) &			
Global - Average± SD (median)	1006.68 ± 1279.09 (412.15)	6.58 ± 1.81 (5.75)	<0.0001
masculine - Mean ± SD (median)	778.66 ± 1233.06 (57.70)	6.95 ± 1.98 (6.5)	<0.0001
Feminine -Mean ± SD (median)	1114.7 ± 1319.11 (606.8)	6.21 ± 1.66 (5.35)	<0.0001
& - non-parametric tests were applied Mann-Whitney, Kruskal-Wallis			

I have studied the factors independent of prediction for different variables - the multiple regression method, for which the following had been taken into account: gender of patients, age, presence/absence of respiratory infections and their type (0 = absent, 1 = acute respiratory infections, 2 = bronchiolitis, 3=pneumonia), presence/absence of respiratory infections history, parental smoking (0 = none, 1 = mother, father = 2, both parents = 3). Overall, the data are presented in table no. 3, where it can be seen that of all the factors studied, respiratory infections are an independent risk factor for serum interleukin 8 (IL-8). In other words, the values obtained for the correlation coefficients suggest a statistically significant correlation only between serum levels of interleukin 8 (IL-8) and positive diagnosis for respiratory infections: acute upper respiratory infections, bronchiolitis, pneumonia.

Table no. 3. Correlation coefficients between IL-8 and demographics, diagnosis, history of respiratory infections, exogenous risk factors in the groups under study

Study group + Control group	IL8
Gender	-
Age	-
Respiratory infections	P=0.0022 coeff=377.74 r=0.450
History of respiratory infections	-
Smoking	-

As one can see, serum interleukin 8 (IL-8) is significantly elevated in respiratory infections. But, following the research, the study of the relationship between IL-8 and types of respiratory infections represented by upper acute respiratory infections, bronchiolitis and pneumonia, there have not been statistically significant differences. The average value of interleukin 8 (IL-8) and standard deviation (SD) in the study group for all the three diagnosis groups of respiratory infections are shown in table no. 4.

Table no. 4. The average value of interleukin 8 (IL-8) and standard deviation (SD) in the diagnosis groups of respiratory infections

INFECTION	Upper respiratory tract infections	BRONCHIOLITIS	PNEUMONIA	P
Global - mean ± SD (median)	714.97 ± 888.45 (502.10)	749.56 ± 1199 (86.6)	1189.34± 1464 (426.9)	ns

Regarding the relationship between serum levels of interleukin 8 (IL-8) and infants' exposure to smoking in the four subgroups: mother (M), father (T), Mother and Father (MF),

without exposure (NO), for the values of IL-8, there were no statistically significant differences (p = 0.047), only between the group with no parental smoking (no exposure - NO) and the group where both parents were smokers (mother and father - MF). Regarding the other subgroups, the differences recorded for the values of interleukin 8 (IL-8) were not statistically significant.

DISCUSSIONS

The multitude of studies conducted for a better understanding of the pathogenesis of acute respiratory infections and of the factors which determine the severity of these diseases has used the model of infection with respiratory syncytial virus (RSV), the most common etiologic agent of acute respiratory infections in infants and toddlers. They showed that all clinical consequences are dependent by the triad: factors that depend on the virus, factors that depend on the host, and by the host immune response. In the infection with RSV, the PMN chemotaxis is dependent by the production of chemokines by respiratory epithelial cells and macrophages. Macrophages or tissue monocytes along with the respiratory epithelial cells are the first cells experiencing RSV at the level of the respiratory tract. In vitro studies have demonstrated that both types of cells respond to infection by cytokine secretion, and both secrete IL-8.(18,19,20)

Recent research on bronchiolitis with RSV in infants has demonstrated a significant association between nasopharyngeal concentrations of interleukin 8 (IL-8) and the severity of the disease. Not surprisingly, in the samples of serum and bronchoalveolar lavage taken from the ventilated infants, there have been found elevated levels of IL-8. Regarding the innate immune response in bronchiolitis with RSV, the importance of interleukin 8 (IL-8) is also underlined by genetic studies that demonstrate the intervention as a determining factor of the severity of the disease of a genetic polymorphism linked to the IL-8 gene.(10,21,22)

The results of this study correlate with current research studies, that consider that measuring interleukin 8 (IL-8) could be a way to assess the infant's innate immune response to acute respiratory infections. Much higher values that I have obtained for serum levels of IL-8 in the infants in the study group compared to values obtained for infants in the control group come to reinforce the idea of using IL- 8 as an immunological marker in acute respiratory tract infections.(21,12,20)

The fact that in acute respiratory infections in infants, there have been elevated serum IL-8, it is another proof for the use of this chemokine as a marker for positive diagnosis. But the study of the mean values and standard deviations for IL-8 in the study group within the three diagnosis groups of respiratory infections: upper respiratory tract infections, bronchiolitis, pneumonia, did not show statistically significant differences.

Although this study has demonstrated once again the usefulness of IL-8 in the positive diagnosis of upper respiratory tract infections, I did not find arguments for using IL-8 as a marker for the differential diagnosis of acute respiratory infections.(10,11,12,23)

Regarding the objective of highlighting a correlation between the harmful behaviour of parents (smoking during pregnancy and lactation), and the acceleration of inflammatory processes expressed by influencing inflammatory serum markers, we can say that in this study, the contribution of IL-8 is supported by statistically significant differences (p = 0.047) which occurred between the group with no parent smoker and the group where both parents were smokers. Exposure to smoking was associated with higher levels of interleukin 8 (IL-8) in the subgroups in which only one parent was a smoker too, but without statistically significant differences between the two groups.(10,19,23)

CONCLUSIONS

In conclusion, interleukin-8 could be an immunological marker in acute respiratory infections in infants and requires further research to determine whether increased serum levels of interleukin-8 in infants with acute respiratory infections is the consequence of immune mechanisms which act as a result of the body's response to the contact with infectious agents, and if breastfeeding is one of the factors that positively influence this process.

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